Short, Cool, and Well Oxygenated – HOPE for Kidney Transplantation in a Rodent Model

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Short, Cool, and Well Oxygenated – HOPE for Kidney Transplantation in a Rodent Model

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Objectives: The aim of this study was to investigate novel and easily applicable preservation perfusion techniques in kidney grafts obtained from donors after circulatory death (DCD).

Background: A novel perfusion approach, hypothermic oxygenated perfusion (HOPE), used for DCD liver grafts, is based on cold perfusion for 1 hour by an oxygenated solution before implantation. Here, we aimed to test HOPE in a rodent model of kidney grafts associated with substantial warm ischemia.

Methods: Rat kidneys were exposed to 30 minutes in situ warm ischemia, without application of heparin. Kidneys were removed and cold stored for 4 and 18 hours, mimicking DCD organ procurement and conventional preservation. In additional experiments, kidneys were normothermically perfused with oxygenated blood for 1 hour after cold storage. In a third group, kidneys were perfused by HOPE for 1 hour after cold storage. In each group, orthotopic kidney transplantation was performed after recipient nephrectomy.

Results: HOPE-treated DCD kidneys showed dramatically better function after transplantation, than cold-stored grafts in terms of nuclear injury, macrophage activation, endothelium activation, tubulus damage, and graft function. A short period of warm oxygenated perfusion before implantation improved graft quality as compared with cold storage, but was significantly less effective in all endpoints compared with HOPE. The effect of HOPE was dependent on perfusate oxygenation in the cold.

Conclusions: HOPE of DCD kidneys was superior to other clinically used preservation approaches, consistent to earlier results in livers. On the basis of this, we assume a strong and generalized effect on solid organ viability by HOPE before transplantation. These results justify a clinical trial.

Keywords: DCD kidney, hypothermic oxygenated perfusion, kidney transplantation, normothermic oxygenated kidney perfusion, survival

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Organ transplantation continues to be limited by a severe shortage of donors. In response to this, there has been a worldwide increase in the use of compromised donors, and a significant proportion of transplanted organs are currently taken from extended criteria donors (ECDs)[1,2] and also donation after circulatory death (DCD).[3] However, these organs generally suffer from a higher risk of dysfunction after implantation, which trigger acute rejection and impair long-term graft survival.[3] Therefore, new concepts of preservation, for example, machine perfusion instead of conventional cold static storage, have been suggested to improve graft viability, differing mainly in perfusate conditions, including temperature and timing of perfusion.[4–7]

In this context, our group developed a short-term 1 to 2-hour Hypothermic Oxygenated Perfusion (HOPE), applied after cold storage in liver transplantation.[8–13] In various animal transplant models, we showed a high protective effect of HOPE.[8–11] In addition, recent human application of HOPE rescued extended DCD livers, exposed to long donor warm ischemia,[12] and was superior than matched, un-perfused DCD liver grafts.[13] On the basis of the success of HOPE in livers, we aimed to test HOPE in kidneys. In a second step, we compared HOPE with a normothermic endischemic perfusion approach, which has recently gained much attention in the UK and Netherlands.[5,6,14,15]

We chose a standardized rodent DCD kidney transplant model, associated with substantial donor warm ischemia, and applied machine perfusion using both techniques against conventional cold storage with subsequent transplantation.

METHODS

Animals

We used male Brown Norway rats in all experiments. The animal ethics committee (009/2015) approved all experiments. Anesthesia during kidney procurement and transplantation was maintained with isoflurane.

Study Design

According to previous publications,[9–11] we opted for a rodent DCD kidney transplant model, with induction of cardiac arrest by incision of the diaphragm without prior heparinization or vessel clamping. We applied 30 minutes of asystolic donor warm ischemia time in all experimental groups and used an established orthotopic model of kidney transplantation for recipients.[16,17] The study analyses the effect of different machine perfusion strategies on graft injury and survival.

The following experimental groups were chosen (Supplementary Fig. 1A, http://links.lww.com/SLA/B16):

(1) Control group: Healthy kidneys (non-DCD) were exposed to minimal cold storage (15 minutes UW solution), and subsequently transplanted (No Injury, n = 10). In additional experiments, kidneys were treated before implantation by either 1-hour normothermic or 1-hour hypothermic perfusion (No injury and perfusion, n = 5 each).
(2) Cold storage group: Nonperfused DCD kidneys were exposed to 30 minutes of in situ warm ischemia and cold stored (CS) for 18 or 4 hours, respectively (UW), and transplanted afterwards (DCD and CS, n = 8/10).

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(3) Cold storage and normothermic perfusion group: DCD kidneys were exposed to 30 minutes of in situ warm ischemia, followed by 18 or 4 hours cold storage along with 1 hour perfusion, and transplanted afterwards (DCD, CS, and normotherm, n = 8/10).
(4) Cold storage and HOPE group: DCD kidneys were exposed to 30 minutes of in situ warm ischemia, followed by 18 or 4 hours cold storage along with 1 hour perfusion, and transplanted afterwards (DCD, CS, and HOPE, n = 8/10).
(5) Cold storage and hypothermic de-oxygenated perfused (HNPE) group: DCD kidneys were exposed to 30 minutes of in situ warm ischemia, followed by 18 or 4 hours of cold storage along with 1 hour perfusion with deoxygenated perfusate (=nitrogenated), and transplanted afterwards (DCD, CS, and HNPE, n = 8/10).

Surgical Technique Donor

Following laparotomy, a period of 30 minutes asystolic warm ischemia (DCD) was induced by bilateral incision of the diaphragm. Consecutive induction of a pneumothorax led to hypoxia and subsequent cardiac arrest, mimicking withdrawal of ventilation in the clinical setting of Maastricht III donors (controlled DCD). The mean time until cardiac arrest was 9.3 ± 2 minutes. Warm ischemia was defined as the time after cardiac arrest to cold flush, corresponding to asystolic warm ischemia. Before retrieval, kidneys were flushed in situ with 6 mL heparinized (1 U/mL) saline at room temperature followed by 5 mL cold UW solution via aorta (renal artery). Kidneys were then excised (weight 0.98 ± 0.078 g) and placed in UW solution (4°C). In the perfusion groups, kidneys received stents for the aorta (renal artery). Ureters of all kidneys were stented (24G).

Surgical Technique Recipient

After machine perfusion, all kidneys were again flushed with 5 mL cold UW solution and a cuff was inserted in the renal vein. Subsequently, kidney transplantation was performed as previously described.[17,18] Before kidney implantation, all recipients underwent bilateral nephrectomy.[16]

Normothermic Perfusion

Normothermic kidney perfusion was performed with a heparinized, leukocyte depleted blood perfusate.[6] Erythrocytes were isolated out of full rat blood and added to Ringers solution, according to Nicholson and Hosgood[6] (hematocrit 20%, leukocyte count <5/mm^3). The perfusate was supplemented by heparin, Mannitol 10%, Dexamethasone, Prostacyclin, Sodium bicarbonate, Nutriflex (B. Braun). Kidneys were perfused continuously and pulsatile (190/min) at 37°C through the renal artery (mean arterial pressure 63 mm Hg, range 55 to 75 mm Hg) by pressure control. Perfusates were actively oxygenated (pO_2 55 to 65 kPa) (Supplementary Fig. 1B, C, http://links.lww.com/SLA/B16).[6,19]

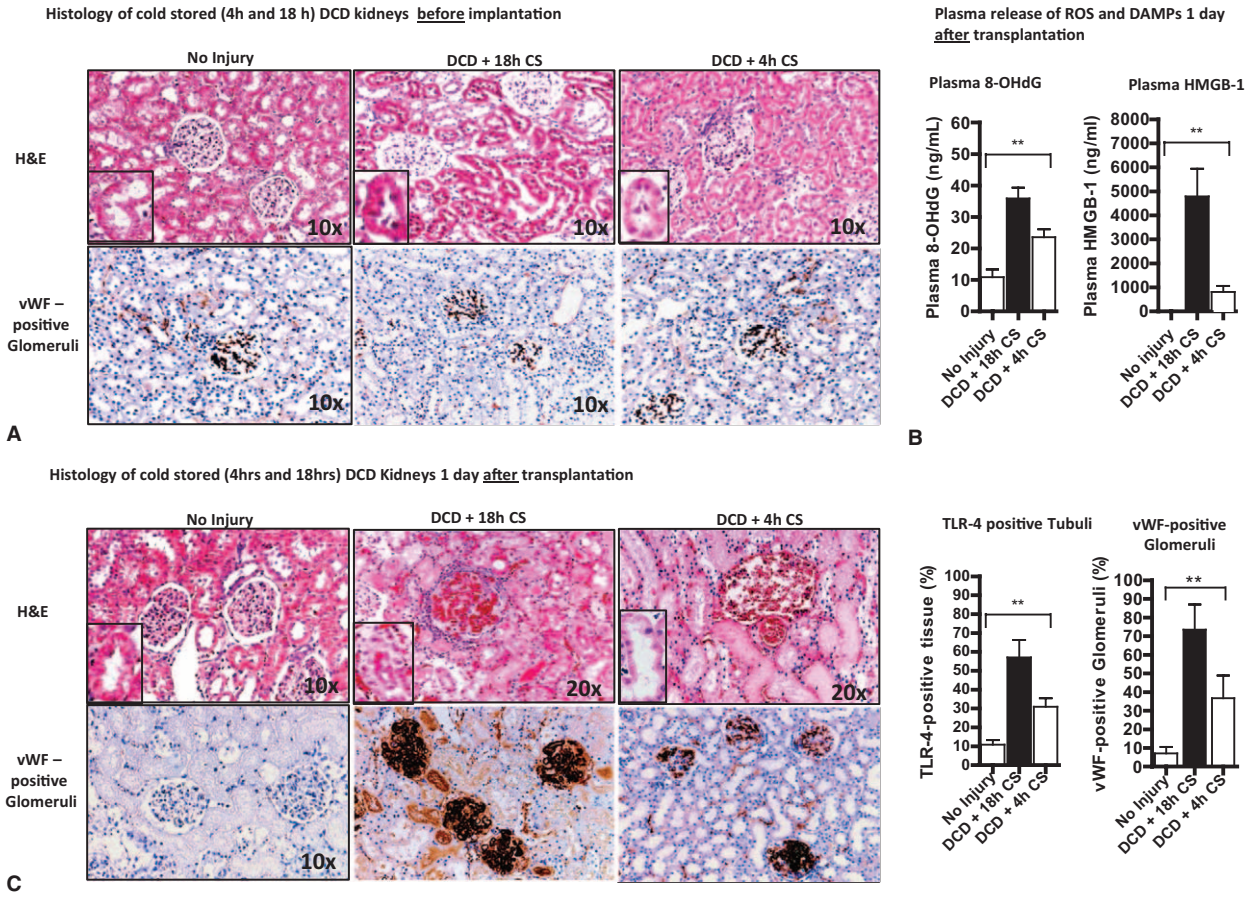

FIGURE 1. Preservation injury before and after transplantation of cold-stored DCD kidneys. Cold-stored (18 hours) DCD kidneys (30 minutes warm ischemia) were microscopically well preserved at the end of cold storage (A). In contrast, following implantation, severe injury occurred in terms of oxidized nuclear DNA (8-OHdG), DAMPs release (HMGB-1), Toll-like receptor activation (TLR-4), and endothelial activation (vWF) (B, C). Reduction of cold storage to 4 hours significantly reduced reperfusion injury (B, C).

Hypothermic Oxygenated Perfusion

HOPE was performed for 1 hour through the renal artery (aortic patch) with a constant perfusion pressure of <18 mm Hg,[20] and active oxygenation (po_2 60 to 80 kPa). We used 50 mL recirculating Kidney-Perfusion Solution-1 (KPS-1) as perfusate. Perfusion box and perfusate were cooled to 4°C by an open bath thermostat (Fig. 1B, C). In additional experiments (group 5), perfusate deoxygenation was facilitated by insufflation of nitrogen resulting in low po_2 (<2 kPa) (HNPE group).[11]

Endpoints

Kidney function and tubular injury were analyzed by NGAL and creatinine in plasma samples. Oxidative damage of DNA was detected in perfusate and plasma by 8-hydroxy-2-deoxy Guanosine (8-OHdG). ATP was measured as previously reported.[9,10] Nuclear injury was measured by release of high mobility group box protein-1. Quantitative real-time polymerase chain reaction (PCR) was performed (*TaqMan* gene expression assays) for Toll-like receptor (TLR)-4, tumor necrosis factor-alpha (TNF-α), high mobility group box-1 protein (HMGB-1), von Willebrand factor (vWF), endothelin 1 (Edn-1), Hepatocyte growth factor. The following staining procedures were performed: Haematoxylin-Eosin (H&E)-staining (tubular injury), TLR-4-staining (Macrophage and tubuli activation), and vWF (endothelial activation). Histological samples were quantified.

Statistical Analysis

Data are presented as mean ± standard deviation (SD). Statistical analysis was performed using the nonparametric Mann-Whitney-Wilcoxon *U* test (GraphPad Prism, version 6.0; San Diego, CA).

RESULTS

Cold Storage of DCD Kidneys

DCD kidneys subjected to 30 minutes in situ warm ischemia and subsequent 18 hours cold storage appeared macro- and microscopically well preserved before implantation with minor tubular cell injury or endothelial cell activation (Fig. 1A) in spite of massive ATP depletion during cold storage (Fig. 2D). After transplantation, we detected severe reperfusion injury in terms of oxidized nuclear DNA (8-OHdG), release of HMGB-1, increased presence of Toll-like receptor-4 (TLR-4) positive cells, endothelial activation (vWF), tubulus injury, and also poor kidney function (Fig. 1B, C). All

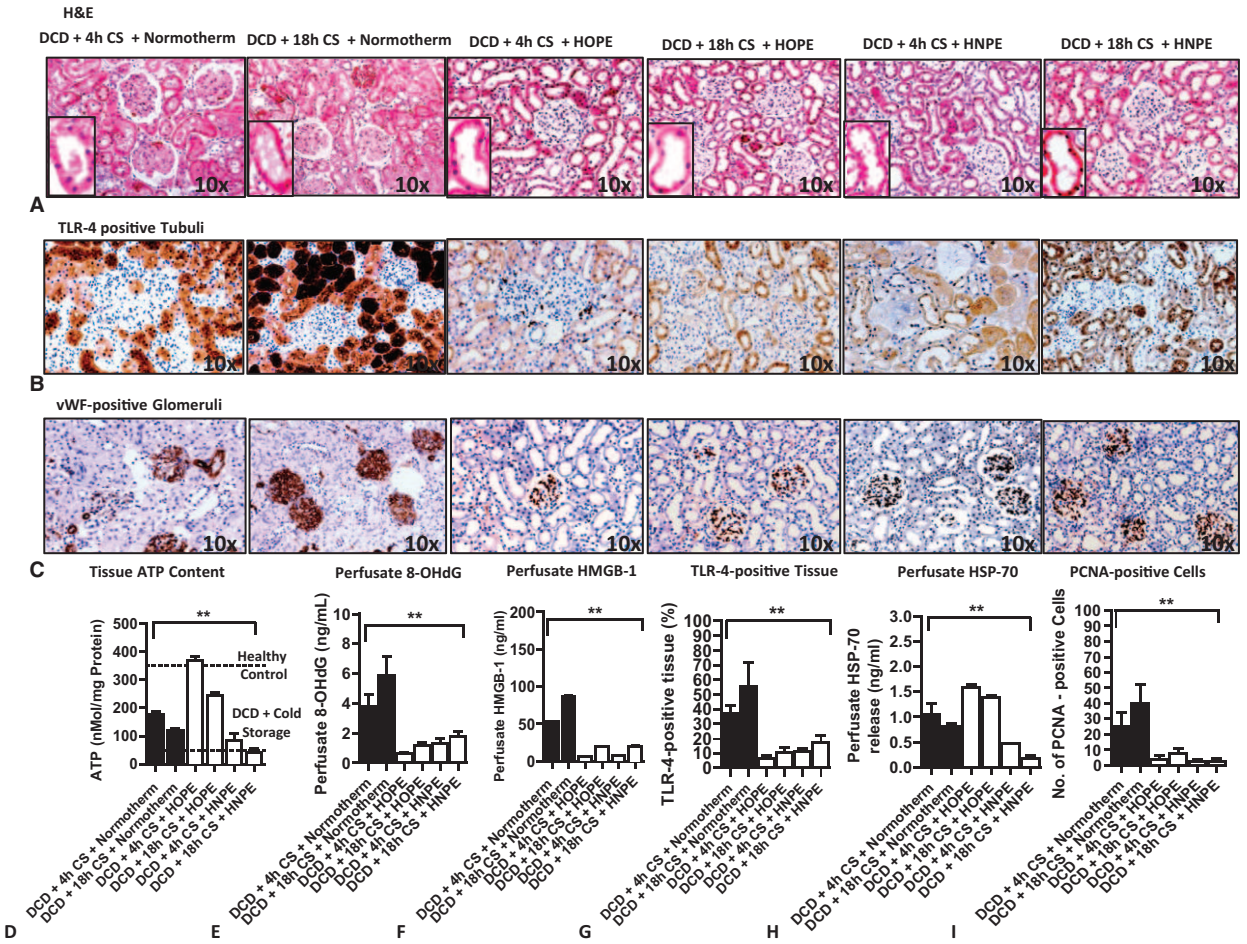

FIGURE 2. Graft injury during 1 hours machine perfusion of cold-stored DCD kidneys (4 and 18 hours). Normothermic perfusion resulted in oxidative stress (E) with release of DAMPs (F), and massive activation of TLR-4 on tubuli (B,G), as well as endothelial cell activation (C) during perfusion. In addition, HSP 70 and PCNA were upregulated during normothermic perfusion (H, I). HOPE treatment reloaded most effectively cellular ATP (D) and caused neither relevant oxidative stress (E), or DAMPs release (F) or TLR-4/endothelial activation (B, C, F, G).

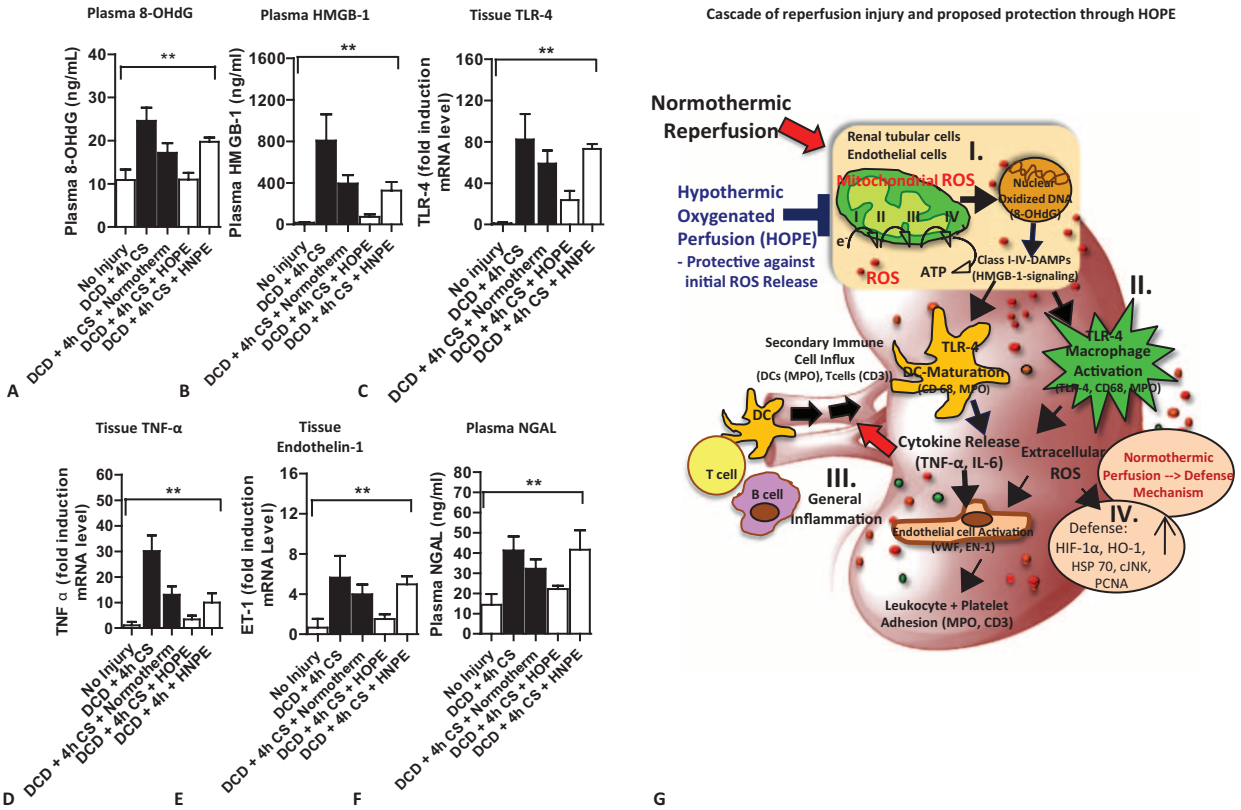

FIGURE 3. Graft injury of cold-stored DCD kidneys 1 day after machine perfusion and transplantation. Normothermic perfused kidney transplants showed less oxidized DNA (A), less HMGB-1 release (B), less tissue TNF-a (D), and less plasma NGAL concentrations (F) compared with cold storage. HOPE treatment was significantly more effective in terms of oxidative stress, DAMPs release, and tubulus/endothelial activation (A to F). Importantly, the benefits of HOPE were abrogated in the absence of perfusate oxygen (HNPE) (A to F). We suggest therefore that mitochondrial repair processes during HOPE are responsible for decreased release of mitochondrial ROS during reperfusion. Due to less oxidative stress, the amount of released DAMPs is subsequently also decreased, together with minor downstream activation endothelial cells (G), similarly as observed in HOPE treated liver grafts.[8]

animals died consecutively within 24 hours of surgery. Reduction of cold ischemia from 18 to 4 hours significantly reduced graft injury in all endpoints (Fig. 1B, C), correlating to recent reports in human,[2,21] but did not prevent from lethal injury in recipients (Fig. 3B).

Cold Storage and Normothermic Oxygenated Perfusion of DCD Kidneys

As a proof of concept, we tested normothermic perfusion by an established technique[5,6] of healthy non-DCD kidneys, which resulted in no relevant injury during perfusion and 100 % graft survival (7 days) after subsequent transplantation (Fig. 3A to C).

In a next step, we treated DCD kidneys after 18 hours cold storage by 1 hour normothermic perfusion. Cellular adenine nucleotide levels increased significantly by normothermic perfusion compared with cold-stored kidneys (Fig. 2D), reflecting successful restart of aerobic metabolism. However, we found high amounts of 8-OHdG and HMGB-1 in the normothermic perfusate (Fig. 2E, F), suggesting relevant oxidative stress during normothermic perfusion. In parallel, we documented a high number of TLR-4 positive cells (Fig. 2B), underlining activation of macrophages and dendritic cells by danger-associated molecular patterns (DAMPs) release during normothermic perfusion. Correspondingly, several antioxidative and regenerative pathways, for example, Heat Shock Protein 70 (HSP 70), proliferating cell nuclear antigen (PCNA), cJNK, were stimulated already during normothermic perfusion due to upregulation of cellular defense mechanisms by reactive oxygen species (ROS) release (Fig. 2H, I). Despite this, all animals died within few hours after subsequent transplantation (18 hours cold storage and 1 hour normothermic perfusion). Reduction of cold ischemia from 18 to 4 hours reduced significantly oxidative stress, DAMPs release, TLR-4, and endothelial activation during normothermic perfusion (Fig. 2). After subsequent implantation, those grafts showed less injury compared with unperfused 4 hours cold-stored DCD kidneys (Figs. 4A to F and 5A to E), but recipients died also within 2 to 4 days (Fig. 3B). These results suggest severe inflammation of cold-stored ischemic (DCD) kidneys already during normothermic perfusion, in contrast to normothermically perfused nonischemic (DBD) kidneys.

Cold Storage and Hypothermic Oxygenated Perfusion of DCD Kidneys

In a third step, we tested our hypothermic perfusion approach, initially developed for livers. DCD kidneys treated after 18 hours cold storage by 1 hour HOPE showed minimal release of DAMPs or reactive oxygen species (8-OHdG) during cold perfusion (Fig. 2E, F) in spite of high oxygen saturation in the perfusate (60 to 80 kPa,

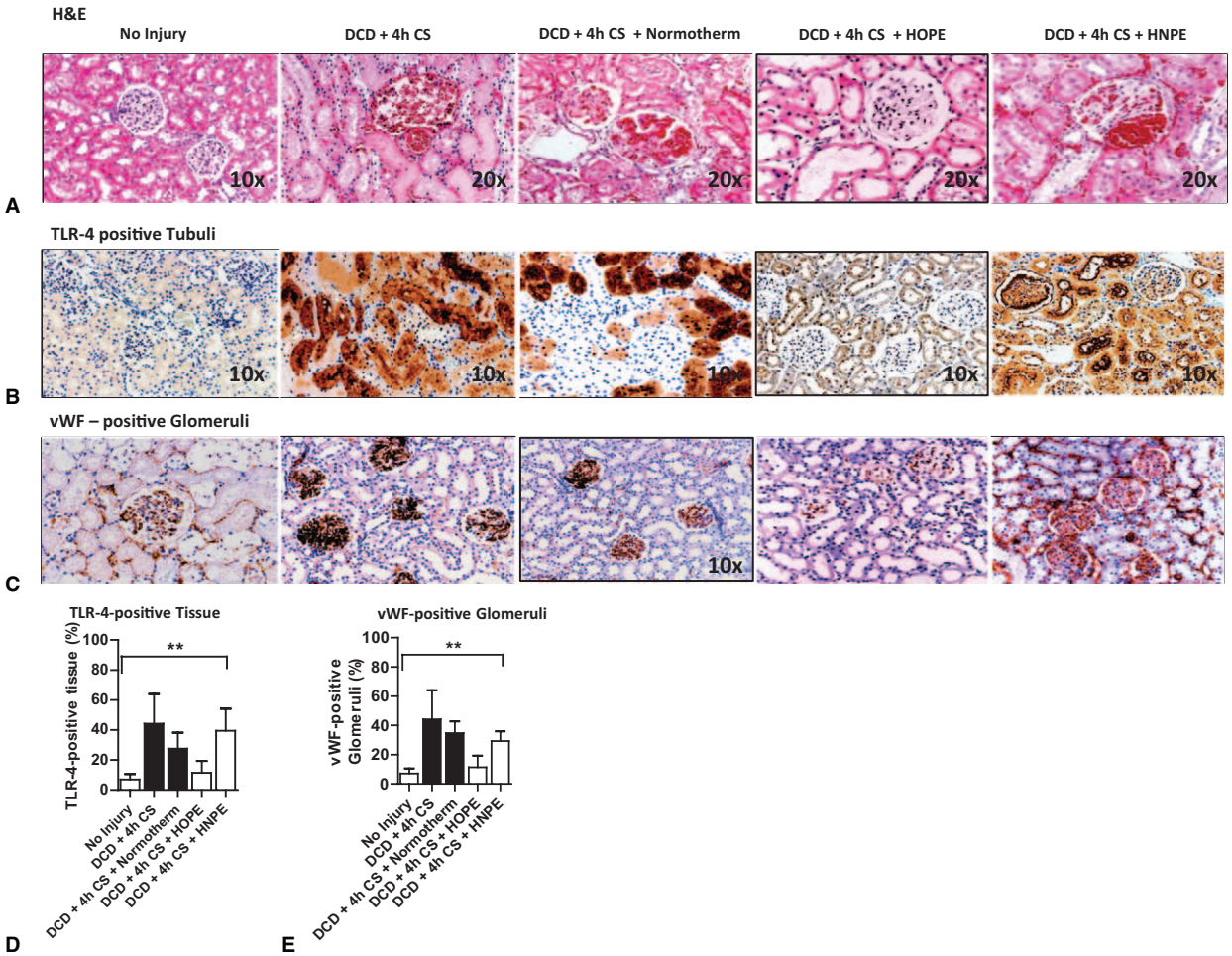

FIGURE 4. Histology of cold-stored DCD kidneys 1 day after machine perfusion and transplantation. Normothermic perfused kidney transplants displayed high activation of TLR-4 positive tubuli (B, D), while HOPE-treated kidney transplants showed low amounts of TRL-4 positive tubuli (B, D), and no staining of vWF in glomeruli, similarly to controls (C, E). Perfusion with deoxygenated perfusate (HNPE) reversed the effects of HOPE (A to E).

Supplementary Fig. 1, http://links.lww.com/SLA/B16). ATP increased significantly and higher by HOPE treatment than normothermic perfusion (Fig. 2D). After implantation, all HOPE-treated kidneys presented with significant less injury compared with cold storage and normothermic perfusion in terms of 8-OHdG- and HMGB-1 release, TRL-4 activation, endothelial activation, and tubulus injury (Figs. 4 and 5). Despite this protection, HOPE treatment after 18 hours cold storage could not reverse lethal injury. However, reduction of cold ischemia from 18 to 4 hours resulted in 80% 7-day survival in contrast to cold storage or normothermic perfusion (Fig. 3B). Histology after 7 days in survivors showed no fibrosis, low endothelial activation, and normal amount of dendritic cells (Fig. 3C).

Cold Storage and Hypothermic Deoxygenated (Nitrogenated) Perfusion of DCD Kidneys

To analyze whether the protective effect of endischemic HOPE relates to the presence of oxygen in the perfusate, we repeated hypothermic perfusion experiments with a deoxygenated perfusate. In contrast to HOPE treatment, kidneys hypothermically perfused without oxygen after cold storage suffered from high release of ROS during implantation, with massive activation of macrophages, dendritic cells, and endothelial cells, similar as seen in cold-stored and nomothermically perfused kidneys (Figs. 4 and 5). All recipients from this experimental group died from graft failure shortly after implantation (Fig. 3A, B).

DISCUSSION

The use of machine perfusion of organs before implantation has several advantages enabling assessment of organ injury, induction of repair mechanisms, or even allowing optional treatment during perfusion. This study, using an established DCD kidney transplantation model, demonstrates 4 important results regarding 2 competitive endischemic machine perfusion approaches, currently discussed for kidneys.

First, HOPE treatment of DCD kidney is superior to normothermic perfusion, and oxygen is the key ingredient during hypothermic perfusion, underlining the importance of aerobic mitochondrial conditioning before normothermic reperfusion. Second, endischemic HOPE treatment of DCD kidneys does not provoke relevant ROS release, in contrast to normothermic perfusion,

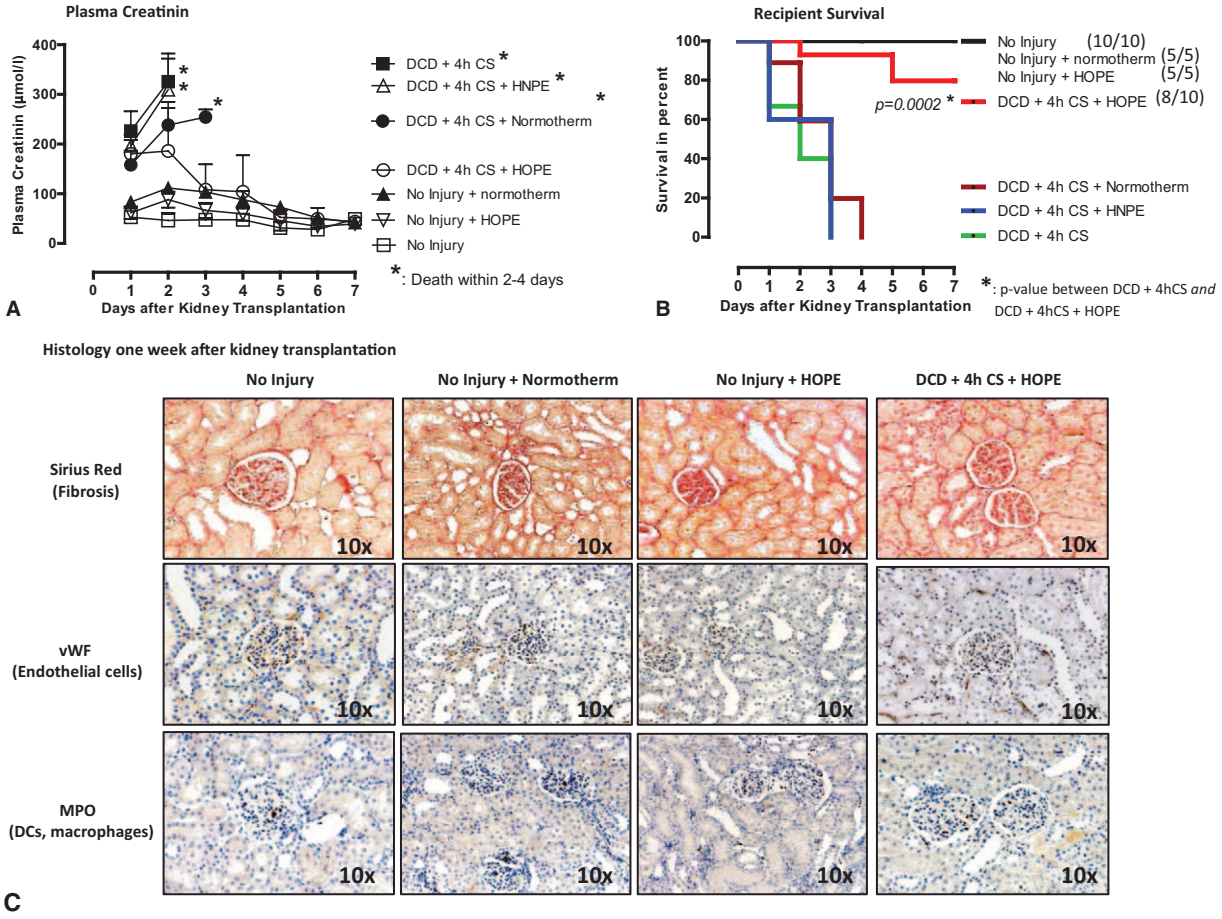

FIGURE 5. Survival and kidney injury during 1 week after kidney TPL. Untreated cold-stored DCD kidney transplants presented with irreversible kidney graft failure 1 to 2 days and death after transplant (A, B). Endischemic normothermic perfusion after cold storage improved slightly plasma creatinine of recipients (A), but all transplanted rats died (B). HOPE-treated DCD kidney transplants also showed higher plasma creatinine levels in the first 2 days after transplants, which however decreased to normal levels within 1 week (B) in 80% of recipients. Histology after 7 days in survivors showed no fibrosis, low endothelial activation, and normal amount of dendritic cells (C).

corresponding to earlier results in livers.[11] Cellular energy charge is significantly higher uploaded than normothermic perfusion. Third, normothermic perfusion of cold-stored DCD kidneys induces an inflammatory milieu during perfusion and triggers reperfusion injury by release of ROS already before implantation. The extent of reperfusion injury during normothermic perfusion depends on the duration of initial donor warm ischemia. Finally, despite induction of reperfusion injury, endischemic normothermic perfusion of cold-stored DCD kidneys is superior to cold storage alone, potentially by induction of cellular defense mechanisms.

In sharp contrast to other solid organs, machine perfusion for human kidneys was clinically introduced already 40 years ago.[22] However, graft survival benefit between pumped and nonpumped standard human kidneys was only 4% in a large randomized trial (94% vs 90% 1 year).[4] In addition, convincing data are lacking, addressing underlying mechanisms of kidney graft protection by machine perfusion.[7] On the basis of this, the overall acceptance of perfusion systems for kidneys has remained relatively poor in many centers, despite the fact that the rate of delayed graft function (DGF) undoubtedly improved by machine kidney perfusion, especially in ECD grafts.[23] Recent studies, however, have triggered a clinical reinterest in kidney machine perfusion, mainly in UK.[5,6,19] These investigators rely on the simple combination of initial cold storage applying normothermic perfusion afterwards for a short period before implantation with ABO-compatible, cross-matched, packed red blood cells.[5,6] Accordingly, current clinical application of this concept in human showed an impressive reduction of DGF after kidney transplantation (6% vs 36%), when treated by 1-hour normothermic perfusion.[6] It was stated that endischemic normothermic perfusion techniques may offer practical advantages and serve as future alternative form of organ preservation.[5] We have developed a similar endischemic perfusion approach for DCD livers in the last 15 years, but using hypothermic oxygenation with acellular perfusate instead of normothermic perfusion with diluted blood.[13,24] We aimed therefore to compare the 2 endischemic machine perfusion approaches in a transplant model with relevant kidney injury, simulating DCD.

Consistent with above-stated studies,[5,6] we found a protective effect of endischemic normothermic perfusion in DCD kidneys, compared with cold storage alone. However, we also show that normothermic perfusion of DCD kidneys induced significant reperfusion injury, evident by high amounts of released ROS, DAMPs, and

endothelial activation. Sustained inflammatory responses are well known to start during any ischemic insult and accelerate upon normothermic reperfusion in vivo.[25] The mechanisms underlying activation of immune cells in the postischemic organ involve DAMPs in conjunction with hypoxia-inducible factors (HIFs) and adhesion molecules.[25] These initiators cause permeability dysfunctions, for example, of the renal vascular endothelium,[26] and trigger release of chemokines, and activate TLRs.[26]

We show in this study that normothermic perfusion of ischemic kidneys induces an inflammatory environment ex vivo as a prerequisite for downstream attracting of macrophages, dentritic cells, and neutrophils (Fig. 4G).[27] In response to this, cellular defense mechanisms are upregulated during normothermic perfusion, similar to ischemic preconditioning, underlined by increased heat shock and HIF-proteins or proinflammatory cytokines such as TNF-α.[28] We would speculate that induction of repair cellular pathways is responsible for protective effects of normothermic perfusion compared with cold storage. Of note, healthy kidneys, not exposed to warm ischemia in donors (controls), showed minor reperfusion injury during normothermic perfusion. These results correspond to earlier investigations regarding normothermic perfusion of nonischemic and ischemic livers.[10]

Our study demonstrates that HOPE prevents oxidative stress or DAMPs release during perfusion.[8,10,24] In addition, HOPE treatment prevents mitochondrial ROS production and downstream macrophage and endothelial activation during reperfusion,[8,29] potentially by a reversible downregulation of mitochondrial electron transfer.[8] Accordingly, kidney grafts treated by HOPE showed significant less TLR-4-positive cells, less TNF-α release, less endothelial cell activation after implantation, and were rescued from lethal injury, in contrast to normothermic perfusion or cold storage alone. These results are in contrast to studies using nonoxygenated cold perfusion at relatively high pressure (30 mm Hg) in pigs,[30] but in line with other previous studies on low pressure oxygenated and endischemic cold kidney perfusion.[31,32] The beneficial effects of HOPE were completely lost when oxygen in the perfusate was replaced by nitrogen, pointing to the key function of perfusate oxygen. Second, we believe that the success of HOPE in kidneys depends strongly also on low pressure conditions (<20 mm Hg), similar to livers,[8] due to a high risk of shear stress and endothelial damage during cold perfusion of kidneys at a perfusion pressure >20 mm Hg.[8,32] On the basis of these observations, the current clinical practice of DCD kidney perfusion may need to be adapted, being most frequently continuous cold perfusion using a pressure of (30/20 mm Hg) LifePort Kidney Transporter (Organ Recovery Systems, Chicago, IL) without active oxygenation.[4,7,31]

Notably, the difference between both perfusion techniques appears multifactorial because of several varying parameters, for example, perfusate composition, temperature, po_2, and perfusion pressure. The results of this study therefore need to be confirmed in human by randomized trials. As a second limitation, we did not investigate upfront normothermic perfusion without any cold ischemia. On the basis of this, starting normothermic perfusion already in donors may lead to different results.[33,34] Furthermore, we cannot exclude that perfusion for >1 hour would have been more effective (warm and cold).

In summary, although normothermic oxygenated perfusion is superior to evaluate online organ metabolism,[35] it also triggers reperfusion injury ex vivo, depending on the extent of donor ischemia. In contrast, HOPE prevents oxidative stress during implantation, possibly by mitochondrial repair mechanism.[36] As these results are highly consistent with data in DCD liver grafts treated by HOPE,[10] we assume that HOPE serves as general novel strategy to address ischemic events in any organs. We, therefore, envision a broad implication of HOPE in the field of transplantation.

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DISCUSSANTS

J. Pirenne (Leuven, Belgium):

Machine perfusion (MP) is a revolution. There is growing evidence that MP is better than static preservation but there is no consensus on duration (continuous or delayed) and temperature (warm or cold) necessary for this protection.

In this article, you show that HOPE a short period of Hypothermic Oxygenated Perfusion after cold storage ameliorates kidneys exposed to warm and cold ischemia. Interestingly, you presented similar data in a liver transplant model at this meeting years ago, showing that HOPE is efficient for both organs.

Original rationale for HOPE (cold/delayed perfusion) is its simplicity. Let us put the simplicity apart for a moment.

HOPE - through active oxygenation - probably works by restoring mitochondrial machinery. There are other data (from Minor) showing that adding O2 to cold storage is beneficial. This is logical since at 4°C there is ongoing metabolism and O2 may prevent partly the switch from aerobic to anaerobic metabolism.

If O2 is ''good'' why would not it be better when given immediately after procurement?

Your warm perfusion group was superior to static storage, but inferior to cold HOPE. I have difficulty to understand that because with warm you have the potential to restore mitochondrial machinery to an even greater extent?

Did you test warm MP immediately after procurement?

Real time assessment of metabolism and function may not be possible in the cold. This is critical because if you want to increase the organ pool you want be able to assess function and viability. Can you comment on that?

Response From P. Dutkowski (Zurich, Switzerland):

Your first question addresses timing of perfusion. In principal, it appears logical to perfuse upfront organs instead of any cold storage, but our results show also that this may not be needed, if cold storage is kept relatively short. In fact, there is no difference between upfront HOPE and HOPE after 6- to 8-hour cold storage in kidneys and livers.

The second question targets the effects of normothermic perfusion on mitochondria. We show in this study that normothermic perfusion of cold stored DCD kidneys initiates an inflammatory reaction that triggers reperfusion injury already before implantation, similarly as seen in normothermic perfused cold stored DCD livers. Of note, this kind of injury is dependent on the extent of donor warm ischemia, and is not observed in DBD grafts. In this series of experiment, we did not test warm perfusion immediately after procurement in DCD kidneys, which may lead to different results.

Your last question was regarding viability assessment during HOPE. Currently, it is feasible to measure graft injury by several markers, but more difficult to measure organ function in the cold. Further research is needed for advanced analysis of mitochondrial metabolism during HOPE, which will however be possible in the future.

T. van Gulik (Amsterdam, The Netherlands):

The step toward upfront continued perfusion using the same methods as in HOPE also seems a logical step toward optimal organ preservation. These organs would be cold perfused from the beginning, apart from when there are logistical constraints with machine perfusion at the site of the donor where the organs are retrieved. Continuous hypothermic oxygenated perfusion for the duration of ischemia is another way to proceed, instead of 1 hour of perfusion.

In your second group of rat kidneys, you used normothermic perfusion at 37-degree celsius. That follows the concept of Lindberg and Carrel who in 1938 pioneered normothermic perfusion of organs. We now think that 37 degrees is actually too warm and we should probably go to sub-normothermic levels. Maybe perfusion at 28 degrees would give better results, even better than HOPE.

Response From P. Dutkowski (Zurich, Switzerland):

I do not fully agree with your first statement, as in our experience, there was no advantage of an immediate cold oxygenated perfusion directly after procurement compared to an endischemic approach after cold storage. Based on this, we are currently more in favor of applying HOPE at the transplant center.

Regarding your second comment, the optimal perfusate temperature for machine perfusion of different organs remains currently unclear. Further research is needed to evaluate this issue.

P.J. Friend (Oxford, United Kingdom):

We are all trying to minimize the acute inflammatory response on reperfusion, and clearly there is substantial benefit in delivering oxygen and repleting ATP stores. Do you feel that the optimal combination here might actually a progressive re-warming starting with your oxygenated cold perfusion and progressing toward the physiological situation, that is, 37 degrees? There are some downsides to cooling and it seems to me that you should be able to optimize the combination in some way.

Response From P. Dutkowski (Zurich, Switzerland):

This is an interesting concept; I do believe that a combination of cold and warm perfusion techniques could further optimize organ function, especially in highly injured organs. This would allow minimizing of early reperfusion injury, for example by initial HOPE, and further evaluation of organ function during subsequent normothermic perfusion.